Antinociceptive Effects of *N*-Acyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine in Mice: Structure–Activity Relation Study of Matrine-Type Alkaloids Part II

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***N*-Acyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridines were synthesized as derivatives of matrine-type alkaloids, and the structure–activity relations were examined by the acetic acid-induced abdominal contraction test. The antinociceptive potencies of *N*-acyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridines were significantly lower than those of (+)-matrine. The antinociceptive effects of *N*-benzyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridines are approximately 5.6 to 6.5 times less than those of *N*-benzoyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine. These findings suggest that the amide group of matrine-type alkaloids is an essential functional group that influences antinociceptive potency. The antinociceptive effect of 4c was markedly antagonized by pretreatment with Naloxone, and that of 3c partially so.**

Key words structure–activity relationship; antinociception; matrine

We reported previously that (+)-matrine **1**, a typical matrine-type lupine alkaloid produced by some *Sophora* plants (Leguminosae), has antinociceptive properties identical to those of pentazocine, and these are mediated mainly through activation of κ-opioid receptors and partially through μ-opioid receptors.[1] (+)-Allomatrine **2**, which is the C-6 epimer of **1**, also has antinociceptive properties, its potency is one-third the potency of **1**. The effects of (+)-allomatrine are mediated only through activation of κ-opioid receptors.[2] The structure–activity relation of this antinociceptive effect is of interest because the structure of the matrine-type alkaloids differs from the structures of conventional κ-opioid receptor agonists such as ethylketocyclazocine, U-50,488,[3,4] and TRK-820[5] (Chart 1). The matrine-type alkaloids may serve as κ-opioid receptor agonist drugs that are more selective and lack the undesirable morphine-like side effects of existing drugs. We reported previously in our study of antinociceptive effects of 1-acyl-4-dimethylaminopiperidines that the amide group of (+)-matrine **1** is essential for antinociceptive potency.[6] The *N*-acyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridines **3a**—**c** and **4a**—**c** were synthesized as derivatives of the matrine-type alkaloids. They included the amide group and the A, B, and C rings of matrine-type alkaloids because they were predicted to comprise the pharmacophore for the antinociceptive effect. *N*-Benzyloctahydropyrido[3,2,1-*ij*]-[1,6]naphthyridines **8** and **9** were made from **3c** and **4c** to confirm the pharmacophore prediction. The antinociceptive effects of these compounds were tested with the acetic acid-induced abdominal contraction test (writhing test) to clarify structure–activity relations.

Synthesis 1-Benzyl-4-piperidone **5** was bisalkylated with acrylonitrile *via* the Stork enamine synthesis.[7] A mixture of bis- and monoalkylated compounds was hydrogenated with 20% palladium hydroxide on carbon to the corresponding amine **6** in 62% yield. We determined that both 3- and 5-cyanoethyl groups were *cis* configuration, because six peaks were observed by ^{13}C-NMR spectra. The amine **6** was acylated with acetic anhydride (**7a**: R=Me, 92%), pentanoyl chloride (**7b**: R=Bu, 94%), and benzoyl chloride (**7c**: R=Ph, 67%) in the presence of triethylamine. Reductive cyclization[8,9] of **7a**—**c** was accomplished with 5% palladium on carbon in glacial acetic acid at room temperature under a hydrogen pressure (5 atm) to give compounds **3a**—**c** in 12 to 19% yields and **4a**—**c** in 8 to 11% yields (Chart 3). We determined the structure of **3a**—**c** and **4a**—**c** by comparing those chemical shifts of 10b position on ^{13}C-NMR spectra. Compounds **3c** and **4c** were reduced with lithium aluminum hydride, to give amines **8** and **9** in 81 and 83% yields, respectively (Chart 4).

Acetic Acid-Induced Abdominal Contraction Assay

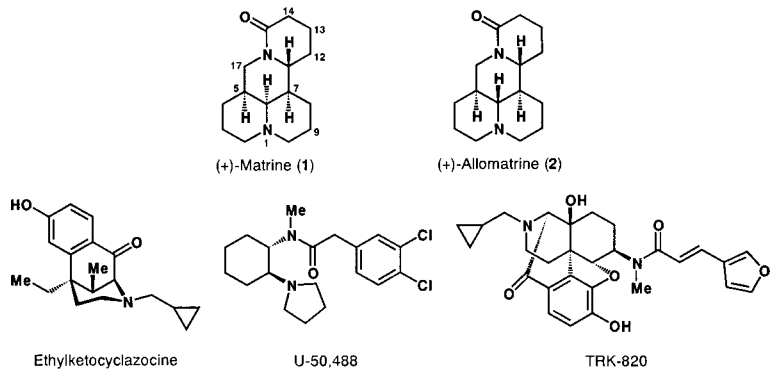

Chart 1

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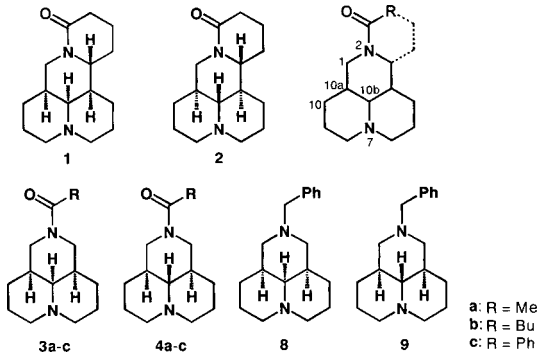
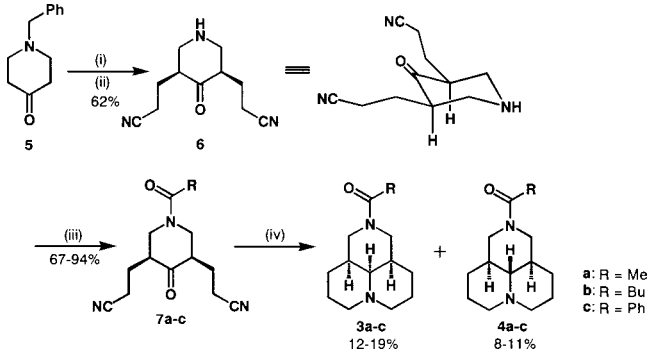
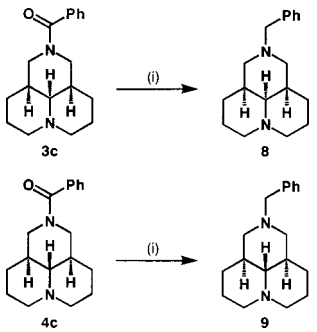


Chart 2



Reagents and reaction conditions: (i) pyrrolidine, benzene, reflux 48 h; (ii) acrylonitrile, EtOH reflux 48 h; (iii) 25% AcOH rt 1 h; (iv) H_2/20% $Pd(OH)_2$–C, EtOH rt 24 h; (v) Ac_2O or RCOCl (R=Bu, Ph), Et_3N, CH_2Cl_2 0 °C 1 h; (vi) H_2/5% Pd–C, AcOH 5 atm rt 48 h.

Chart 3



Reagents and reaction conditions: (i) $LiAlH_4$, THF reflux 2 h.

Char 4

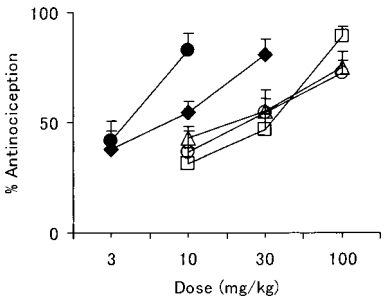


Fig. 1. Dose–Response Lines for the Antinociceptive Effect of s.c. Administration of (+)-Matrine **1** (●), (+)-Allomatrine **2** (◆), **3a** (○), **3b** (△), **3c** (□) in the Acid-Induced Abdominal Contraction Assay in Mice

Each point represents the mean±S.E. of 10 mice.

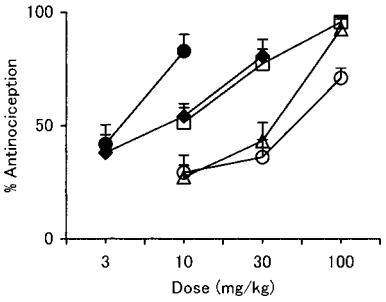


Fig. 2. Dose–Response Lines for the Antinociceptive Effect of s.c. Administration of (+)-Matrine **1** (●), (+)-Allomatrine **2** (◆), **4a** (○), **4b** (△), **4c** (□) in the Acid-Induced Abdominal Contraction Assay in Mice

Each point represents the mean±S.E. of 10 mice.

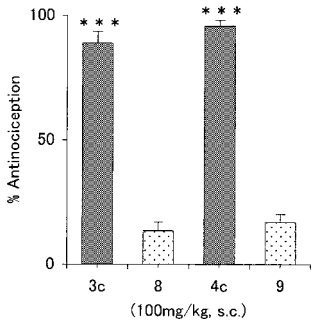


Fig. 3. The Antinociceptive Effect of s.c. Administration of Amide Compounds **3c** and **4c** in the Acid-Induced Abdominal Contraction Assay in Mice

*** $p<0.001$ *versus* the corresponding amine compounds **8** and **9**. Each column represents the mean with S.E. of 10 mice in each group.

(Writhing Test) and Data Analysis For writhing test and data analysis refer to our previous work.[6] Opioid receptor antagonist Naloxone (1 mg/kg, i.p.) was dissolved in saline, and administered 30 min before each drug.

RESULTS

Compounds **3a**—**c** (doses of 10—100 mg/kg, s.c.) produced dose-dependent inhibition of the writhing response in mice (Fig. 1). The ED_{50} values (mg/kg with 95% confidence limits) were 35.6 mg/kg (−127.9—199.2) for **3a**, 24.7 mg/kg (−81.8—171.3) for **3b**, and 37.5 mg/kg (15.7—59.3) for **3c**. Compounds **4a**—**c** (doses of 10—100 mg/kg, s.c.) also produced dose-dependent inhibition of the writhing response in mice (Fig. 2). The ED_{50} values (mg/kg with 95% confidence limits) were 56.0 mg/kg (25.5—86.5) for **4a**, 40.5 mg/kg (28.4—52.5) for **4b**, and 8.6 mg/kg (0.7—107.1) for **4c**. All drugs with the amide group produced dose-dependent inhibition of the writhing response in mice. The antinociceptive potencies of **8** and **9** (100 mg/kg) were significantly lower than those of the corresponding amides **3c** and **4c** (Fig. 3).

The antinociceptive effect of **4c** was markedly antagonized by pretreatment with Naloxone, and that of **3c** was partially antagonized (Fig. 4).

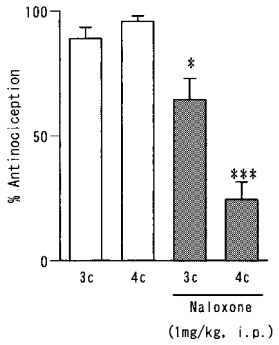


Fig. 4. Effects of Naloxone on the Antinociceptive Effects of **3c** and **4c** (100 mg/kg, s.c.) in mice

Naloxone (1 mg/kg) was injected i.p. 30 min before the injection of **3c** and **4c**. Each column represents the mean with S.E. of 10 mice in each group. $*p<0.05$, $***p<0.001$ *versus* the respective group.

DISCUSSION

The present experiments show that compounds **3a**—**c** and **4a**—**c**, which include the amide group and the A, B, and C rings of matrine-type alkaloids, produced dose-dependent antinociception in mice. The antinociceptive effects of **3c** and **4c** are approximately 5.6 to 6.5 times greater than those of **8** and **9**. These findings showed that the amide group of (+)-matrine **1** is essential for antinociceptive potency.

The antinociceptive effect of **4c** was markedly antagonized by pretreatment with naloxone. It was clarified that allomatrine derivative **4c** produced the antinociceptive effect mainly through the activation of opioid receptor, while that of **3c** was partially antagonized by pretreatment with naloxone. Like the above, it was determined that matrine derivative **3c** produced the antinociceptive effect partially through the activation of opioid receptor.

The antinociceptive potency of compounds **3c** and **4c**, which contain a benzoyl group, is greater than that of the other synthesized compounds **3a**—**b** and **4a**, **b**. Thus, matrine derivatives with greater potencies may be developed by improving the lipophilicity with an acyl group. The antinociceptive potencies of **3a**—**c** and **4a**—**c** are greater than those of the 1-acyl-4-dialkylaminopiperidines, which have only the C ring of **1**.[5] It is conceivable that the antinociceptive potency of the latter compounds depends on the lipophilicity of the molecule and on the distance of the amide from the tertiary amino groups. We obtained some useful information regarding structure–activity relations in this study, but we failed to identify any compound with an antinociceptive potency greater than that of **1**.

MATERIALS AND METHODS

General High-resolution MS (HR-MS) were measured at 70 eV using a direct inlet system on a JEOL D300 spectrometer. ^{1}H-NMR (270 MHz) and ^{13}C-NMR (67.8 MHz) spectra were recorded using tetramethylsilane (TMS) as an internal standard with a JEOL JNM-LA270 spectrometer. IR spectra were measured with a JASCO FT/IR-200 spectrometer. Column chromatography was carried out on Silica gel 60 (100—210 μm, Kanto Chemical Co., Inc.), Wako gel C-300 (45—75 μm, Wako Pure Chemical Industries, Ltd.), and Lichrospher Si 60 (40—63 μm, Merck).

***cis*-3,5-Dicyanoethyl-4-oxo-piperidine, 6** A mixture of the piperidone **5** (10.02 g, 52.94 mmol) and pyrrolidine (15.30 g, 215.12 mmol) in benzene (50 ml) was refluxed with a dean-stark trap for 48 h. The solvent was evaporated and the residue was dissolved in anhydrous EtOH (50 ml). Acrylonitrile (11.20 g, 226.16 mmol) was added to the solution and the reaction mixture was refluxed for 48 h. The solvent was evaporated, and the residue was dissolved in 25% AcOH (50 ml) and stirred at room temperature for 1 h. The solution was extracted with CH_2Cl_2 and the extract was washed with 5% HCl and 5% NaOH. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to give the crude product, which was difficult to purify. To a solution of the crude product in EtOH (50 ml) was added $Pd(OH)_2$ on carbon (2.0 g, 20% w/w), hydrogenated at atmospheric pressure at room temperature for 24 h. Catalyst was filtered off, and solvent was evaporated. The residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH/28% NH_4OH=100 : 9 : 1) to give the title compound as a pale yellow oil (6.74 g, 32.84 mmol, 62%): ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.55 (2H, m), 1.97 (1H, s), 2.03—2.16 (2H, m), 2.45—2.51 (4H, m), 2.57—2.65 (4H, m), 3.49 (2H, dd, J=6.9, 12.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 15.0, 22.3, 51.1, 54.0, 119.7, 210.0. IR (Film) cm^{-1}; 1700 (C=O), 2240 (C≡N), 3330 (NH). HR-MS *m/z*: Found 205.1191 (Calcd for $C_{11}H_{15}N_3O$ 205.1215).

***cis*-1-Acetyl-3,5-dicyanoethyl-4-oxo-piperidine, 7a** To a solution of the amine **6** (5.36 g, 26.11 mmol) and triethylamine (10.22 g, 101.0 mmol) in CH_2Cl_2 (25 ml), cooled to 0 °C under nitrogen, was added acetyl chloride (3.98 g, 50.70 mmol). The reaction mixture was stirred at 0 °C for 2 h, then sat. K_2CO_3 was added, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with 10% HCl, dried over anhydrous (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_4OH=100 : 9 : 1) and gave the title compound as a pale yellow oil (5.91 g, 23.90 mmol, 92%): ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.70 (2H, m), 1.99—2.21 (2H, m), 2.22 (3H, s), 2.45—2.75 (7H, m), 3.08 (1H, dd, J=12.0, 13.2 Hz), 4.17 (1H, ddd, J=2.8, 6.0, 13.2 Hz), 4.96 (1H, ddd, J=2.8, 6.0, 12.1 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.0, 20.5, 21.3, 21.6, 45.5, 47.1, 47.5, 50.0, 118.8, 168.2, 207.1. IR (Film) cm^{-1}; 1640 (amide C=O), 1710 (C=O), 2240 (C≡N). HR-MS *m/z*: Found 248.1415 (M+1) (Calcd for $C_{13}H_{18}N_3O$ 2248.1399).

***cis*-1-Pentanoyl-3,5-dicyanoethyl-4-oxo-piperidine, 7b** As described for the preparation of **7a**, the amine **6** (2.90 g, 14.13 mmol) was acylated with pentanoyl chloride (2.46 g, 20.40 mmol) and triethylamine (4.31 g, 42.59 mmol) in CH_2Cl_2 (15 ml), and purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_4OH=180 : 9 : 1): colorless crystal, yield 3.86 g (94%); mp 93 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, J=7.3 Hz), 1.34—1.48 (2H, m), 1.56—1.73 (4H, m), 2.11—2.18 (4H, m), 2.41—2.73 (7H, m), 3.04 (1H, t, J=12.4 Hz), 4.21 (1H, d, J=12.4 Hz), 4.98 (1H, d, J=10.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 13.2, 14.4, 21.6, 21.7, 21.7, 26.6, 32.0, 46.0, 47.5, 48.0, 49.6, 118.8, 171.0, 207.3. IR (KBr) cm^{-1}; 1630 (amide C=O), 1710 (C=O), 2240 (C≡N). HR-MS *m/z*: Found 289.1791 (Calcd for $C_{16}H_{23}N_3O_2$ 289.1790).

***cis*-1-Benzoyl-3,5-dicyanoethyl-4-oxo-piperidine, 7c** As

described for the preparation of **7a**, the amine **6** (4.18 g, 20.36 mmol) was acylated with benzoyl chloride (4.36 g, 31.02 mmol) and triethylamine (6.21 g, 61.37 mmol) in CH_2Cl_2 (20 ml), and purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_4OH=180 : 9 : 1): colorless crystal, yield 4.36 g (67%); mp 100 °C. (^{1}H-NMR was unanalyzable by the rotation obstacle of Ph group.) ^{13}C-NMR ($CDCl_3$) δ: 14.0, 21.6, 46.5, 47.5, 49.7, 51.4, 118.7, 126.2, 128.0, 129.7, 134.0, 169.5, 207.1. IR (KBr) cm^{-1}; 1640 (amide C=O), 1720 (C=O), 2240 (C≡N). HR-MS *m*/*z*: Found 309.1503 (Calcd for $C_{18}H_{19}N_3O_2$ 309.1477).

all *trans*-2-Acetyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 3a, all *cis*-2-Acetyloctahydropyrido[3,2,1-*ij*][1,6]-naphthyridine, 4a Palladium on carbon (1.5 g, 5% w/w) was added to a solution of dinitrile **7a** (3.06 g, 12.37 mmol) in AcOH (240 ml) and the mixture was hydrogenated at 4.5 atm and room temperature for 48 h. The suspension was filtered off, and the solvent was evaporated. The residue was diluted with CH_2Cl_2, and the organic phase was washed with aqueous NaOH (10% w/v). The organic phase was dried over anhydrous $NaSO_4$ and evaporated, and the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH/28% NH_4OH=140 : 9 : 1) to give matrine-type derivative **3a** as a pale yellow oil (319 mg, 1.43 mmol, 12%) and allomatrine-type derivative **4a** as a pale yellow oil (223 mg, 1.03 mmol, 8%).

3a: ^{1}H-NMR ($CDCl_3$) δ: 1.43—1.79 (10H,m), 1.90—2.00 (1H, m), 2.09 (3H, s), 2.09—2.11 (2H, m), 2.79 (2H, d, *J*=12.7 Hz), 3.01 (1H, dd, *J*=12.5, 12.9 Hz), 3.32 (1H, ddd, *J*=1.7, 4.3, 12.9 Hz), 3.57 (1H, t, *J*=12.9 Hz), 4.24 (1H, ddd, *J*=1.7, 4.3, 12.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 20.9, 21.1, 27.4, 27.5, 35.2, 36.3, 40.7, 46.0, 56.7, 56.8, 62.6, 168.3. IR (Film) cm^{-1}; 1645 (amide C=O). HR-MS *m*/*z*: Found 222.1715 (Calcd for $C_{13}H_{22}N_2O$ 222.1732).

4a: ^{1}H-NMR ($CDCl_3$) δ: 0.92—1.02 (2H, m), 1.31 (1H, t, *J*=9.6 Hz), 1.39—1.51 (2H, m), 1.65—1.73 (6H, m), 1.98—2.03 (2H, m), 2.08 (3H, s), 2.21 (1H, dd, *J*=12.2, 13.2 Hz), 2.77 (1H, dd, *J*=12.2, 13.1 Hz), 2.86 (2H, d, *J*=11.3 Hz), 3.65 (1H, ddd, *J*=2.3, 3.8, 13.2 Hz), 4.56 (1H, ddd, *J*=2.3, 3.8, 13.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 21.4, 24.5, 28.0, 28.1, 39.2, 40.1, 46.4, 51.5, 56.1, 56.2, 71.2, 168.6. IR (Film) cm^{-1}; 1640 (amide C=O). HR-MS *m*/*z*: Found 222.1707 (Calcd for $C_{13}H_{22}N_2O$ 222.1732).

all *trans*-2-Pentanoyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 3b, all *cis*-2-Pentanoyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 4b As described for the preparation of **3a** and **4a**, the dinitrile **7b** (2.93 g, 10.12 mmol) was hydrogenated with Pd–C (2.0 g, 5% w/w) in AcOH (200 ml), and purified by silica gel chormatography (CH_2Cl_2/MeOH/28% NH_4OH=140 : 9 : 1): yielded matrine-type derivative **3b** as a pale yellow oil (517 mg, 1.96 mmol, 19%) and allomatrine-type derivative **4b** as a pale yellow oil (298 mg, 1.13 mmol, 11%).

3b: ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, m), 1.29—1.70 (14H, m), 1.95 (2H, dd, *J*=10.7, 11.4 Hz), 2.18 (1H, br), 2.32 (2H, t, *J*=8.7 Hz), 2.79 (2H, d, *J*=10.7 Hz), 3.00 (1H, dd, *J*=12.5, 13.0 Hz), 3.37 (1H, d, *J*=9.4 Hz), 3.54 (1H, dd, *J*=12.4, 12.5 Hz), 4.26 (1H, d, *J*=13.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 13.3, 20.6, 21.8, 26.9, 27.1, 32.1, 35.0, 36.1, 40.3, 44.7, 56.3, 62.2, 170.1. IR (Film) cm^{-1}; 1630 (amide C=O). HR-MS *m*/*z*: Found 264.2219 (Calcd for $C_{16}H_{28}N_2O$ 264.2202).

4b: ^{1}H-NMR ($CDCl_3$) δ: 0.88—1.03 (5H, m), 1.27—1.45 (5H, m), 1.54—1.68 (8H, m), 1.9—2.02 (2H, m), 2.20 (1H, dd, *J*=4.13 Hz), 2.33 (2H, dt, *J*=3.8, 7.5 Hz), 2.72 (1H, dd, *J*=11.4, 13.3 Hz), 2.86 (2H, d, *J*=11.5 Hz), 3.70 (1H, ddd, *J*=2.3, 3.5, 13.3 Hz), 4.58 (1H, ddd, *J*=2.3, 3.5, 13.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 13.4, 21.8, 24.1, 26.8, 27.5, 32.1, 38.7, 39.6, 45.8, 50.0, 55.5, 70.6, 170.1. IR (Film) cm^{-1}; 1645 (amide C=O). HR-MS *m*/*z*: Found 264.2215 (Calcd for $C_{16}H_{28}N_2O$ 264.2202).

all *trans*-2-Benzoyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 3c, all *cis*-2-Benzoyloctahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 4c As described for the preparation of **3a** and **4a**, the dinitrile **7c** (3.90 g, 12.61 mmol) was hydrogenated with Pd–C (2.0 g, 5% w/w) in AcOH (130 ml), and purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_4OH=140 : 9 : 1): yielded matrine-type derivative **3c** (520 mg, 1.83 mmol, 15%) and allomatrine-type derivative **4b** (380 mg, 1.34 mmol, 11%) as colorless crystals.

3c: mp 115 °C. (^{1}H-NMR was unanalyzable by the rotation obstacle of Ph group.) ^{13}C-NMR ($CDCl_3$) δ: 21.1, 27.5, 27.6, 35.4, 36.6, 41.5, 47.2, 57.0, 62.9, 126.6, 128.2, 129.2, 136.3, 170.0. IR (KBr) cm^{-1}; 1630 (amide C=O). HR-MS *m*/*z*: Found 284.1873 (Calcd for $C_{18}H_{24}N_2O$ 284.1889).

4c: mp 100 °C. (^{1}H-NMR was unanalyzable by the rotation obstacle of Ph group.) ^{13}C-NMR ($CDCl_3$) δ: 23.8, 27.2, 38.6, 39.5, 46.3, 51.9, 55.3, 70.4, 126.0, 127.6, 128.7, 135.3, 169.0. IR (KBr) cm^{-1}; 1630 (amide C=O). HR-MS *m*/*z*: Found 284.1908 (Calcd for $C_{18}H_{24}N_2O$ 284.1889).

all *trans*-2-Benzyldecahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 8 To a solution of lithium aluminum hydride ($LiAlH_4$) (59 mg, 1.55 mmol) in tetrahydrofuran (THF) (2 ml) was added the solution of the amide **3c** (0.32 mg, 1.13 mmol) in THF (1 ml), and then refluxed under nitrogen atmosphere for 2 h. The reaction mixture was quenched with water, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2, washed with saturated K_2CO_3 and brine, dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/28% NH_4OH=190 : 9 : 1), gave matrine-type derivative **8** as a pale yellow oil (0.25 g, 81%). ^{1}H-NMR ($CDCl_3$) δ: 1.26—1.54 (6H, m), 1.66—2.03 (7H, m), 2.38—2.55 (4H, m), 2.77 (2H, dm, *J*=10.9 Hz), 3.53 (2H, s), 7.22—7.34 (5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 21.9, 28.2, 29.3, 35.7, 53.4, 57.3, 63.1, 63.3, 126.7, 128.0, 129.0, 138.9. HR-MS *m*/*z*: Found 270.2106 (Calcd for $C_{18}H_{26}N_2$ 270.2096).

all *cis*-2-Benzyldecahydropyrido[3,2,1-*ij*][1,6]naphthyridine, 9 As described for the preparation of **8**, the amide **4c** (0.34 g, 1.20 mmol) was reduced with $LiAlH_4$ (60 mg, 1.56 mmol), and purified by silica gel column chromatography (CH_2Cl_2/MeOH/28% NH_4OH=90 : 9 : 1): colorless crystal, yield 0.27 g (83%); mp 82 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (1H, ddd, *J*=4.9, 11.9, 11.9 Hz), 0.94 (1H, ddd, *J*=4.9, 11.9, 11.9 Hz), 1.10 (1H, t, *J*=9.1 Hz), 1.53—1.77 (10H, m), 1.94 (2H, ddd, *J*=4.1, 11.2, 11.2 Hz), 2.76 (1H, dm, *J*=9.4 Hz), 2.83 (1H, dm, *J*=11.2 Hz), 3.46 (2H, s), 7.20—7.31 (5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 24.8, 28.6, 39.2, 56.3, 59.2, 62.9, 71.5, 126.8, 128.1, 129.0, 138.3. HR-MS *m*/*z*: Found 270.2150 (Calcd for $C_{18}H_{26}N_2$ 270.2096).

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